REMARKS

I. Status of the Claims

Claims 1-3 are pending in the application, claims 1 and 2 are provisionally rejected for alleged obviousness-type double-patenting, and claim 3 is rejected under 35 U.S.C. §112, first paragraph. The specific grounds for rejection are set forth in detail below.

II. Obviousness-Type Double-Patenting

Claims 1 and 2 are provisionally rejected under the judicially-created doctrine of obviousness-type double-patenting. Given the provisional nature of the rejection, applicants submit that the first application to be otherwise allowable should be passed to issue, and the rejection made non-provisional in the remaining application.

III. Rejection Under 35 U.S.C. §112, First Paragraph

Claim 3 is newly rejected as lacking an enabling disclosure. According to the examiner, the absence of *in vivo* data, coupled with the complex nature of the biochemical pathways involved and the issues relating to delivery of nucleic acids to living subjects, render the claims non-enabled. Applicants traverse.

First, it is argued that, in view of Ghivizzani *et al.* and other references showing transfection efficiency is relatively low for *in vivo* by non-viral gene delivery approaches, one of skill in the art would be forced to conduct undue experimentation to achieve the claimed invention. However, Ghivizzani *et al.* summarized the opportunities in the treatment of rheumatoid arthritis for gene delivery strategies in which the cells are ***transfected with a transgene and forced to express that transgene***. This approach is completely different from the

approach of the present invention, which employs decoy oligonucleotides, which are short double-stranded oligo-deoxyribonucleotides of only a length up to 30 bases. Thus, the decoy oligonucleotides do not have to be transfected into a cell and expressed from a vector, but instead are readily taken up by cells as already discussed in detail in the response of April 13, 2007, and in the declaration of Dr. Markus Hecker filed with that response. Moreover, the cells treated with a decoy oligonucleotide according to the present invention are not forced to express ***any*** transgene. Decoy oligonucleotides are readily taken up by the target cell and simply bind to transcription factors therein.

In addition to not facing the hurdles ascribed to Ghivizzani, the present invention utilizes technology that has been successfully demonstrated with other kinds of decoy oligonucleotides. Indeed, injection of decoys into knee joints of mice have been shown to have a high impact on the treatment of said knee joint. For example, Hückel *et al.* describe the application of STAT-1 decoy oligonucleotides into knee joints of mice (see, *e.g.*, page 3, paragraph entitled "*Treatment*") and the positive effects on antigen-induced arthritis (see, *e.g.*, page 5, paragraph entitled "*Clinical effects of STAT-1 decoy oligonucleotide treatment on antigen-induced arthritis*"). Thus, it is evident from these *in vivo* mouse experiments that decoys are highly suitable for the treatment of diseases, *e.g.*, for the treatment of arthritis by direct injection into the knee.

Moreover, the examiner argues that there is an insufficient link between the biochemical pathways being altered by the decoy in cells *in vitro* and the effects representative of therapy in a person. In response, applicants direct the examiner to the examples in the specification of the present application, which clearly demonstrate the effect of the claimed decoy oligonucleotide to restore the effectiveness of IL-10 in human endothelial cells from humans with $^{-786}$C/C-genotype.

Namely, the examples in the specification show that IL-10 increases the eNOS-expression in endothelial cells from humans with $^{-786}$T/T-genotype (see FIG. 4A). In contrast, the eNOS-expression in endothelial cells from humans with $^{-786}$C/C-genotype is not increased by IL-10 (see FIG. 4B). Thus, IL-10 is ineffective in endothelial cells from humans with $^{-786}$C/C-genotype.

The reasons for the ineffectiveness of IL-10 in endothelial cells in humans with $^{-786}$C/C-genotype is that an inhibitory transcription factor binds at position –786 of the eNOS gene in endothelial cells from humans with $^{-786}$C/C-genotype, but not in endothelial cells from humans with $^{-786}$T/T-genotype. Thus, the transcription factor inhibits the induction of the eNOS-expression by IL-10 in endothelial cells from humans with $^{-786}$C/C-genotype, but not from humans with $^{-786}$T/T-genotype. The decoy oligonucleotide according to the present invention binds to the inhibitory transcription factor and thus prevents the binding of the inhibitory transcription factor at position –786 of the eNOS gene in endothelial cells from humans with $^{-786}$C/C-genotype. Accordingly, the induction of the eNOS-expression by IL-10 is no longer inhibited by the inhibitory transcription factor.

Thus, IL-10 is able to induce the eNOS-expression in endothelial cells from humans with $^{-786}$C/C-genotype after the treatment of said cells with the decoy oligonucleotide according to the present invention. This correlation is proven in the examples of the present invention. A direct effect of the increase in eNOS-expression is the inhibition of the CD154-induced new synthesis of IL-12 in the endothelial cells (see FIG. 5 of the present application). Thus, the induction of the eNOS-expression by IL-10 inhibits the synthesis of IL-12 in endothelial cells. Since IL-10 is ineffective in endothelial cells from humans with $^{-786}$C/C-genotype, no inhibition of the IL-12 synthesis is to be expected in such cells after the addition of IL-10. This is confirmed by the bars called CD154 and CD154+IL-10 in FIG. 6 of the specification. But, after the treatment of such

cells with a decoy oligonucleotide according to the present invention, IL-10 is clearly effective in inhibiting the synthesis of IL-12 (see, *e.g.*, Table 3 of the present application).

Thus, to summarize, the examples from the instant specification (shown in FIG 4A, FIG 4B, FIG 5 and FIG 6) clearly demonstrate that IL-10 is ineffective in endothelial cells from humans with $^{-786}$C/C-genotype, but becomes effective after the treatment of such cells with decoy oligonucleotides according to the present invention.

An additional comment on the absence of *in vivo* data is as follows. The data obtainable from human endothelial cells cannot currently be confirmed in *in vivo* models for the simple reason that no animal model with a $^{-786}$C/C-genotype exists. The $^{-786}$C/T-polymorphism of the eNOS-gene is a phenomenon occurring ***only*** in humans and not in any other mammals. Humanized mice carrying the corresponding promoters in front of the endogenous mouse gene are being engineered, but it will require another 2 years before such animals can be subjected to appropriate challenge experiments.

However, as described above the examples of the present invention clearly show that IL-10 is ineffective in endothelial cells from humans with $^{-786}$C/C-genotype, but becomes effective after the treatment of such cells with decoy oligonucleotides according to the present invention. Thus, the decoy oligonucleotide according to the present invention allows IL-10 to induce a number of biochemical reactions. Since there are currently no animal models available in which decoy oligonucleotides according to the present invention can be tested, another model has to be used. This can be accomplished in that the effect of IL-10 is determined by the absence or the presence of IL-10 - the absence of IL-10 can be used as a model for a $^{-786}$C/C-genotype in which the biochemical reactions being normally induced by IL-10 are inhibited. The presence of IL-10 can be used as a model for a $^{-786}$C/C-genotype in which IL-10 is able to induce biochemical

reactions, since the decoy oligonucleotides prevent the inhibitory transcription factors to inhibit the biochemical reactions induced by IL-10. Consequently, the influence of the decoy-oligonucleotide can be modeled *in vivo* by the comparison of IL-10-deficient mice with non-IL-10-deficient mice, namely wild-type mice.

In fact, the influence of IL-10 on arthritis has been investigated in the art *in vivo* by the comparison of IL-10-deficient mice and wild-type mice. For example, Finnegan *et al.* describes the induction of arthritis by the immunization with collagen in IL-10-deficient mice and wild-type mice. The result is, that:

(i) IL-10-deficient mice have an earlier onset of the arthritis than wild-type mice (see page R20, Fig. 1(a)).

(ii) IL-10-deficient mice were in contrast to wild-type mice still arthritic at the time of termination of experiments (see page R20, Fig. 1(a)).

(iii) Arthritis severity in IL-10-deficient mice was significantly exacerbated in comparison with that of wild-type mice (see page R20, Fig. 1(b)).

Thus, *in vivo* tests clearly confirm the positive effect of IL-10 on arthritis. Since the examples of the present invention provide clear evidence that the decoy oligonucleotide according to the present invention neutralizes the inhibiting factors for IL-10, there is, in contrast to the examiner's opinion, a clear link between the biochemical pathway being evidently altered by the decoy oligonucleotide in human endothelial cells and the effects representative of therapy *in vivo*, namely in mice.

The same holds true for the positive influence of IL-10 on coronary heart disease. Coronary heart disease is caused by coronary atherosclerotic lesions. It has been shown that IL-

10 plays *in vivo* a critical role in both atherosclerotic lesion formation and stability. For example, Mallat *et al.* describes the investigation of IL-10 deficient and wild-type mice fed with an atherogenic diet. IL-10-deficient mice had a 3-fold higher risk of getting atherosclerotic lesions as compared to wild-type mice (see, *e.g.*, abstract of Mallat *et al.*). Moreover, it has been shown that after the transfer of murine IL-10 to IL-10-deficient mice having atherosclerotic lesions, a 60% reduction in atherosclerotic lesion size was achieved (see, *e.g.*, abstract of Mallat *et al.*). Thus, a clear link between the biochemical pathway being altered by the decoy oligonucleotide in human endothelial cells and the effects representative of therapy *in vivo*, namely in mice, are confirmed for coronary heart disease as well.

Moreover, the following additional observations are provided:

1. Recent publications confirm that rheumatoid arthritis and coronary heart disease must have a genetic basis on which both diseases develop. For example, Table 1 of van Halm *et al.* confirms that the prevalence of coronary heart disease is clearly increased in humans having rheumatoid arthritis in comparison to humans having no rheumatoid arthritis.
2. The results of Potteaux *et al.* demonstrate that IL-10 expressed by leukocytes prevents exaggerated advanced atherosclerosis development and plays a critical role in modulation of cellular and collagen plaque composition (see, *e.g.*, abstract). Also Caligiuri *et al.* suggest that IL-10 deficiency plays a deleterious role in atherosclerosis (see, *e.g.*, abstract).
3. Fichtlscherer *et al.* suggest a correlation between serum levels of IL-10 and improved NO bioavailability (see, *e.g.*, page 48, first para).

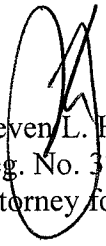
4. Rossi *et al.* concluded that C allele at the $^{-786}$C/T endothelial nitric oxide synthase polymorphism is associated with a higher risk of multivessel coronary artery disease in Caucasians (see, *e.g.*, abstract of Rossi *et al.*).
5. Colombo *et al.* provide evidence that the $^{-786}$C/T-polymorphism of the eNOS gene is associated with the presence and severity of angiographically defined coronary artery disease in the Italian population and that those individuals carrying both eNOS variants simultaneously might have a higher risk of developing coronary artery disease (see, *e.g.*, abstract).
6. Cattaruzza *et al.* confirm that the $^{-786}$C/T-polymorphism constitutes a genetic risk factor for coronary heart diseases (see, e.g., abstract).
7. Melchers *et al.* confirm that individuals with the $^{-786}$C/C genotype have an increased risk of developing rheumatoid arthritis (see, *e.g.*, abstract).

Together, these data in the specification and the published information above show that: (a) the $^{-786}$C/C genotype results in reduced IL-10 activity, and this leads to an increase incidence of both coronary heart disease and rheumatoid arthritis; (b) decoy oligonucleotides of the present invention restore IL-10 activity in human cells having this genotype; and (c) the ability to transfer decoy oligonucleotides of the present invention *in vivo* has been demonstrated in other contexts, and does not suffer from the limitations in the art cited by the examiner. Thus, it is respectfully submitted that the claims are indeed enabled. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

IV. Conclusion

In light of the foregoing, applicants respectfully submit that all claims are in condition for allowance, and an early notification to that effect is earnestly solicited. The examiner is invited to contact the undersigned attorney at (512) 536-3184 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,



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